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Contribution to the knowledge of green frog populations (*Rana esculenta* complex, Anura, Amphibia) of the Kis-Balaton Landscape Protection Area, Hungary

by
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Abstract: Biometrical and serological properties of 172 green frogs from three sampling sites were examined, in order to determine the population structure of water frogs. According to electrophoretic phenotypes of LDH-B (lactate-dehydrogenase) intermediate migrating *lessonae* allele (L_i) and slow migrating *ridibunda* allele (L_s) were the most common. Fast (R_f) and slow (R_s) migrating *ridibunda* alleles were not found in homozygous form and only one sample manifested them in heterozygous form. LT/CIL and DP/CIL were the most adequate morphometric ratios for morphological discrimination. Morphological and biochemical data suggest, that there was a L-E population system in the Kis-Balaton Landscape Protection Area during the years 1989-1990. These results are in contrast with *lessonae-esculenta-ridibunda* mixed population investigated earlier.
Key words: *Rana esculenta* complex, population structure, morphological and biochemical discrimination, Kis-Balaton Landscape Protection Area

INTRODUCTION

Central-European green frogs are usually divided into three forms, which originally had been described as *Rana esculenta* Linnaeus, 1758, *Rana ridibunda* Pallas, 1771 and *Rana esculenta* var. *lessonae* Camerano, 1882.

There was a controversy among herpetologists with regard to the taxonomic status of these forms (see GÜNTHER 1987, GUBÁNYI 1990 for reviews).

Experimental hybridizations (BERGER 1967, 1968, 1970, GÜNTHER 1973, 1978, BINKERT *et al.* 1982), biochemical and karyological investigations (TUNNER 1970, 1972, GÜNTHER 1975, UZZEL & BERGER 1975) indicate that there are two biologically good species, the marsh frog (*Rana ridibunda* Pall.) and the pool frog (*Rana lessonae* Cam.). The edible frog (*Rana esculenta* L.) represents their hybridogenetic hybrid coexisting with one of the parental species or forming pure populations in which triploid specimens are frequent. On the other hand the three forms of water frogs are more seldom found together (BORKIN 1986, GUBÁNYI 1988, LŐW *et al.* 1989, GÜNTHER 1983).

Considering these features DUBOIS & GÜNTHER (1982) proposed a new evolutionary systematic category, klepton (*Rana klepton esculenta*), belonging to a third taxonomic category distinct from both the categories "species" and "subspecies".

In consequence of its hybrid origin (BERGER 1973, DUBOIS & GÜNTHER 1982, UZZEL *et al.* 1977) *Rana esculenta* is genetically heterogeneous and has intermediate morphological character between the two parental species. For

this reason morphological ratios are generally used for distinguishing (GÜNTHER 1973, 1975, BERGER 1971, HOTZ & UZZEL 1982, BORKIN 1979).

Current morphological examinations, biochemical and karyological experiments (GUBÁNYI 1988, 1990, LŐW *et al.* 1989; MÉSZÁROS & BARTOS 1978) cannot present satisfactory data about Hungarian green frog populations.

The scope of this study was to investigate the LDH isozyme patterns and the morphological characteristics of green frogs in the Kis-Balaton Landscape Protection Area during the years 1989-1990.

MATERIAL AND METHODS

Water frogs from three sampling sites of the Kis-Balaton Landscape Protection Area were used for this study: 1. Vörs, frogs were collected in an alder forest (*Cariceto elongatae Alnetum*) situated in a depression; 2. Catch brain of the Kis-Balaton Reservoir, few km from Zalavár; 3. Diás island, frogs were captured nearby the experimental station, which had already been investigated in 1987 (GUBÁNYI 1988, LŐW *et al.* 1989), only in 1990. Ten morphometric characters were included into the analyses: body length (LC), head width (LTC), internarial distance (SPI), nostril to anterior eyelid commisure (DNO), snout to nostril (DIN), intercanthal distance (SPCR), tibia (LT), femur (LF), first toe length (DP), inner metatarsal tubercle length (CIL). From these measurements 7 additional ratios were computed for morphological analyses (LC/LT, LC/LF, LC/CIL, LC/DP, LF/LT, LT/CIL, CIL/DP). Frogs were identified by means of electrophoretic phenotype of LDH-B isozymes. Blood sampling was done from the hindlimb of the frogs and were centrifuged (5 min, 4500 rpm) and stored at 25 °C until processing (GUBÁNYI *et al.* 1991). Polyacrilamide gel electrophoresis was carried out after MAURER (1971) and gels were incubated in darkness at 38 °C in a mixture of 0.3 mg/ml NAD, 0.125 mg/ml NBT, 0.06 mg/ml PMSO and 2.5 mg/ml calcium-lactate in 0.05 M Tris-HCL buffer of pH 8.0. The significance of differences was evaluated by using the Mann-Whitney U-test (HOTZ & UZZEL 1982).

RESULTS

Considering electrophoretic phenotype referring to the B locus of lactate-dehydrogenase, species distribution of the three sampling area showed marked differences (Fig. 1). Two alleles (L_i, L_f) were observed in *Rana lessonae*, which occurred in heterozygous form, too (Table 1). The most balanced frequency of these two alleles was presented in individuals from the Diás island. At the same time in *Rana esculenta* specimens the slow migrating *ridibunda* allele (R_s) was more frequent than the fast migrating *ridibunda* allele (R_f). L_i/R_s heterozigotes dominated at all the three sampling sites and in Diás island this was the only pattern of R_s.

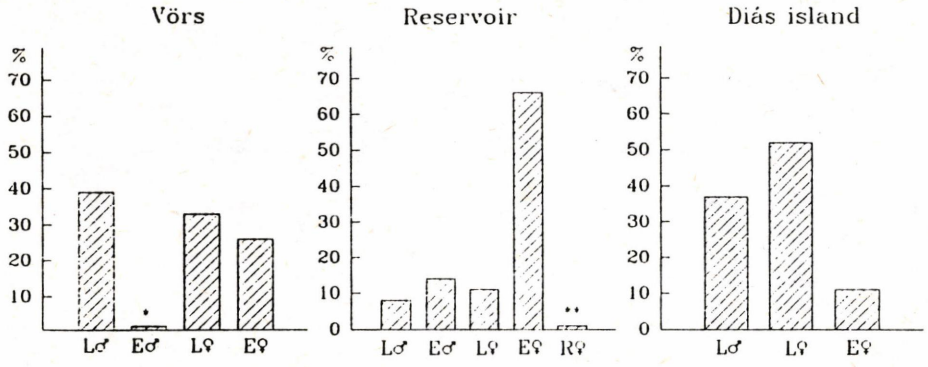

Fig. 1. Species distribution of green frogs according to the electrophoretic phenotypes of LDH-B isozymes in the Kis-Balaton Landscape Protection Area. (*R. lessonae* = L; *R. esculenta* = E; *R. ridibunda* = R; * = 2 %; ** = 1 %)

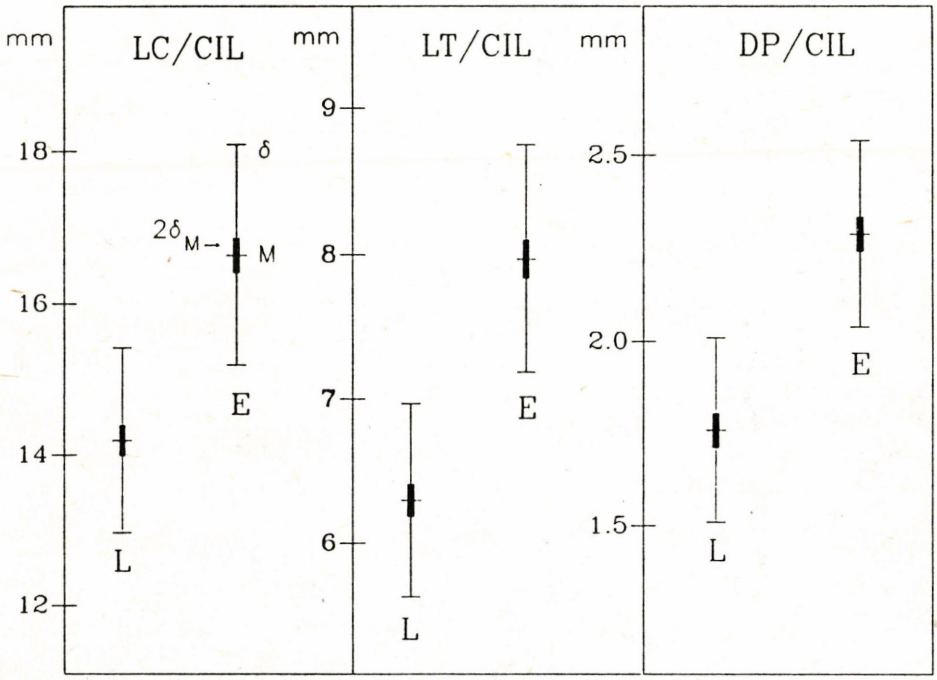

Fig. 2. Vertical diagram of LC/CIL, LT/CIL and DP/CIL indices for *R. lessonae* (L,n=94) and *R. esculenta* (E,n=77) for pooled samples. (M=mean, δ =standard deviation, 2δn=standard error)

Table 1. Allelic frequencies of LDH-B isozymes in samples of *Rana lessonae, R. esculenta* and *R. ridibunda* from three sampling sites at the Kis-Balaton Landscape Protection Area. Sample size is given in parenthesis

	R. lessonae		*R. esculenta*				*R. ridibunda*	
	L_i	L_f	L_i	L_f	R_s	R_f	R_s	R_f
Vörs	0.63	0.37	0.36	0.14	0.32	0.18	–	–
	(39)		(15)					
Kis-Balaton Reservoir	0.75	0.25	0.38	0.12	0.46	0.04	0.50	0.50
	(14)		(57)				(1)	
Diás island	0.56	0.44	0.50	–	0.50	–	–	–
	(41)		(5)					

Li = intermediate mobility lessonae allele; Lf = fast mobility lessonae allele; Rs = slow mobility ridibunda allele; Rf = fast mobility ridibunda allele.

The proportion of male edible frogs (*Rana esculenta* L.) was very low in the sample from Vörs and it contained mostly male and female *R. lessonae* and female *R. esculenta* specimens (Fig. 1). Female individuals of edible frogs composed more then 60 percentage of the animals raised from the Kis-Balaton Reservoir. On the other hand one per cent of all the specimens was *R. ridibunda* at this area. In the sample of Diás island a lot of pool frogs were found. There were no male specimens identified as *R. esculenta* or *R. ridibunda* at this area.

As Table 2 shows the morphological measurements of *R. esculenta* and *R. lessonae* overlap each other (The number of *R. esculenta* specimens found in Vörs and Diás island was not enough to be examined statistically). The scale of overlap is smaller for males or it can be neglected regarding body length (LC), nostril to anterior eyelid commisure (DNO) and intercanthal distance (DIN). On the other hand females are hardly separated from one another. Significant sexual dimorphism occurred in the morphometric characters in our samples, especially in *R. lessonae* captured in Vörs and Diás island and in *R. esculenta* raised from Kis-Balaton Reservoir .

Table 2. Summary of variation in ten measurements of adult female and male water frogs collected from three sampling sites at the Kis-Balaton Landscape Protection Area. Upper line: arithmetic means and standard deviations; lower line: significance of the difference between males and females (Mann-Whitney U test). * = difference significant at 95 % confidence level. ** = difference significant at 99 % confidence level. Sample sizes are given in parentheses.

	Rana lessonae				*Rana esculenta*			
	Vörs		Reservoir		Diás island		Reservoir	
	Males(21)	Females(18)	Males(6)	Females(8)	Males(17)	Females(24)	Males(10)	Females(47)
LC	46.9 ± 4.9	53.0 ± 5.7	57.5 ± 3.0	63.6 ± 7.3	47.9 ± 3.3	53.1 ± 5.7	64.9 ± 3.3	73.7 ± 10.3
	0.001**		0.107		0.000**		0.001**	
Ltc	15.9 ± 1.9	17.6 ± 2.5	19.6 ± 1.1	21.2 ± 2.8	15.9 ± 0.8	17.6 ± 2.5	21.5 ± 1.4	24.2 ± 3.8
	0.064		0.228		0.000**		0.006**	
SPI	3.7 ± 0.2	3.9 ± 0.5	4.2 ± 0.2	4.6 ± 0.7	4.0 ± 0.3	3.9 ± 0.5	4.4 ± 0.2	5.1 ± 0.6
	0.234		0.081		0.001**		0.000**	
SPCR	6.3 ± 0.6	6.9 ± 0.8	7.3 ± 0.4	8.2 ± 0.9	6.9 ± 0.4	6.9 ± 0.8	8.4 ± 0.9	9.1 ± 1.2
	0.022*		0.042*		0.000**		0.050*	
DNO	3.6 ± 0.3	3.9 ± 0.5	3.9 ± 0.3	4.5 ± 0.4	3.5 ± 0.2	3.9 ± 0.5	4.7 ± 0.2	5.4 ± 0.6
	0.011*		0.020*		0.000*		0.000**	
DIN	4.1 ± 0.5	4.4 ± 0.5	4.7 ± 0.4	5.3 ± 0.9	4.4 ± 0.3	4.4 ± 0.5	5.5 ± 0.2	6.1 ± 0.9
	0.023*		0.107		0.000**		0.006**	
LF	21.9 ± 2.2	25.0 ± 2.9	27.5 ± 1.8	29.7 ± 3.3	23.0 ± 1.6	25.0 ± 2.9	32.3 ± 1.4	35.4 ± 4.9
	0.000**		0.181		0.000**		0.004**	
LT	20.9 ± 2.3	24.2 ± 2.9	26.2 ± 1.9	28.7 ± 3.1	21.6 ± 1.1	24.2 ± 2.9	31.9 ± 1.3	35.4 ± 4.9
	0.000**		0.141		0.000**		0.001**	
CIL	3.3 ± 0.3	3.6 ± 0.5	4.1 ± 0.4	4.3 ± 0.7	3.5 ± 0.2	3.6 ± 0.5	3.9 ± 0.3	4.4 ± 0.6
	0.025*		0.282		0.000**		0.009**	
DP	5.8 ± 0.8	6.8 ± 0.9	7.1 ± 0.6	8.1 ± 1.2	5.8 ± 0.4	6.8 ± 0.9	9.3 ± 0.7	10.1 ± 1.3
	0.000**		0.081		0.000**		0.013*	

LC = Body length; Ltc = Head with; SPI = Internarial distance; SPCR = Intercanthal distance; DNO = Nostril to anterior eyelid commisure; DIN = Snout to nostril; LF = Femur; LT = Tibia; CIL = Inner metatarsal tubercle length; DP = First toe length.

Sexual dimorphism was found in four morphological ratios (LC/LT, LC/LF, LC/DP, LF/LT) out of the seven calculated, too (Table 3). The analysis of the pooled sample of the three sampling sites showed differences between *R. esculenta* and *R. lessonae* regarding the means and standard deviations of two of the sexually not dimorphic ratios (LT/CIL,DP/CIL) (Fig. 2). Because of the extreme values, these indices couldn't give sufficient information for discriminating between *R. lessonae* and *R. esculenta* individuals in 9.6-12.9 and 9.9-13.0 per cent of cases, respectively.

Table 3. Summary of variation in seven ratios of adult female and male water frogs collected from three sampling sites at the Kis-Balaton Landscape Protection Area. Upper line: arithmetic means and standard deviations; lower line: significance of the difference between males and females (Mann-Whitney U test). * = difference significant at 95 % confidence level. ** = difference significant at 99 % confidence level. Sample sizes are given in parentheses.

	Rana lessonae				*Rana esculenta*			
	Vörs		Reservoir		Diás island		Reservoir	
	Males(21)	Females(18)	Males(6)	Females(8)	Males(17)	Females(24)	Males(10)	Females(47)
LC/LT	2.2±0.1	2.2±0.1	2.2±0.0	2.2±0.2	2.2±0.0	2.2±0.ö	2.0±0.9.	2.1±0.1
	0.099		0.572		0.016*		0.208	
LC/LF	2.1±0.1	2.1±0.0	2.1±0.0	2.1±0.2	2.1±0.0	2.1±0.1	2.0±0.1	2.1±0.1
	0.665		0.345		0.078		0.024*	
LC/DP	8.2±0.6	7.8±0.7	8.2±0.6	7.9±1.1	8.2±0.5	7.8±0.7	6.9±0.4	7.3±0.4
	0.064		0.949		0.289		0.031*	
LC/CIL	14.2±0.8	14.9±1.6	14.2±0.9	15.0±1.4	13.6±1.1	14.9±1.6	16.6±1.3	16.9±1.5
	0.245		0.282		0.483		0.651	
LF/LT	1.0±0.0	1.0±0.0	1.0±0.0	1.1±0.0	1.1±0.0	1.0±0.1	1.0±0.1	1.0±0.0
	0.727		0.345		0.172		0.023*	
LT/CIL	6.4±0.4	6.8±0.9	6.4±0.4	6.8±1.1	6.1±0.4	6.8±0.9	8.1±0.7	8.1±0.8
	0.202		0.949		0.435		0.785	
DP/CIL	1.7±0.1	1.9±0.3	1.7±0.1	1.9±0.4	1.6±0.1	1.9±0.3	2.4±0.3	2.3±0.2
	0.073		0.949		0.791		0.275	

LC/LT = Body length/Tibia; LC/LF = Body length/Femur; LC/DP = Body length/First toe length; LC/CIL = Body length/Inner m. t. length; LF/LT = Femur/Tibia; LT/CIL = Tibia/Inner m. t. length; DP/CIL = First toe length/Inner m. t. length.

DISCUSSION

The hybrid origin of *Rana esculenta* and its coexistence with one of the parental species or both of them make the identification of green frogs even more difficult. Because of morphological measurements and strict positive correlation among length measurements one generally applies morphometric ratios. The indices DP/CIL and LT/CIL have been used in the last three decades for morphological discrimination (see Introduction). The LC/CIL and the two ratios mentioned above didn't show sexual dimorphism in our samples from the Kis-Balaton Landscape Protection Area. This result was similar to BERGER's (1966) and BORKIN's (1979) investigations. The upper value of the standard deviation in DP/CIL and LT/CIL indices in *R. lessonae* agreed with the value of the identification keys suggested by ENGELMANN *et al.* (1985) for distinguishing between *R. lessonae* and *R. esculenta*. Thus 12.9 and 9.6 per cent of pool frog individuals were proved to be extremes. On the other hand 9.9 and 13.0 per cent of edible frogs had similar features.

Morphological measurements of frogs captured in Kis-Balaton Reservoir (Table 2) showed larger values than the others. This may be attributed to the productivity of the biotope, predation and cannibalism that occurred among *R. esculenta* individuals.

The LDH-B isozyme patterns have been intensively investigated in different parts of Europe (UZZEL & BERGER 1975 for review). Based on the results of the years 1989-1990 L_i and R_s alleles were the most common. This was different from the allele frequency of the investigated L-E or R-E populations. For LDH, two alleles (L_i,L_s) were common in *R. lessonae* in these three samples. L_i was more common. It occurred at frequencies between 0.56-0.76 (Table 1). The corresponding frequencies in *R. esculenta* specimens varied between 0.36-0.50. On the other hand the third *lessonae* allele (L_s) was not manifested in our samples. R_f allele, which is very common in Poland and Germany, was present less frequently and only in heterozygous forms with *lessonae* alleles in this region. But one individual consisted of R_f and R_s *ridibunda* alleles in heterozygous form, which also was morphologically *R. ridibunda* in the Kis-Balaton Reservoir.

The L-E population system suggested by present data for the three sample sites at the Kis-Balaton Landscape Protection Area don't agree with the results found in Diás island in 1987 (GUBÁNYI 1988, LŐW *et al.* 1989). The R_s and R_f *ridibunda* alleles had been determined in both homozigous and heterozygous form, while R_s *ridibunda* allele occurred only in heterozygous form with L_i *lessonae* allele in female *R. esculenta* specimens in later Diás island samples. On the other hand male *R. esculenta* at this sampling area was not found in 1990. But they might exist in the River Zala and in canals situated nearby. If we take the results of earlier investigation into consideration it seems, that the mixed *lessonae-esculenta-ridibunda* population may be formed either by contacting of two different biotopes or by mating of *R. esculenta* individuals among themselves, which results in infertile *R. ridibunda* offsprings. The former idea is supported by the investigation of DELY at the River Zala in 1951 (G.O. DELY, pers. comm.), who identified adult *R. esculenta* and *R. ridibunda* specimens along the river bank.

Different distribution of *R. lessonae* and *R. esculenta* (Fig. 1) among sampling sites points out, that anthropogenic effects may influence the population structure of water frogs in favour of *R. esculenta*. BERGER (1987) had the same findings in Poland.

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